Enzyme Kinetics? Elementary, my dear ...

Kinetics of Enzyme-Catalysed Reactions

Desirazu N Rao

Enzymes, the catalysts of biological systems, remain at the heart of all living systems. As Cornish Bowden aptly remarked, understanding enzymes means understanding catalysis, and catalysis is concerned with kinetics. This article briefly describes a few straightforward rules which are sufficient to describe the basic reaction kinetics of most enzymes.

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Most of the chemical reactions that occur in biological systems are catalysed by proteins called enzymes. These reactions occur at physiological temperatures (37°C), pressures and pH values , unlike similar chemically catalysed reactions which often require much harsher conditions. For instance, at a temperature of 550°C and a pressure of about 250 atmospheres, finely powdered iron is used as a catalyst to fix atmospheric nitrogen. In contrast, certain bacteria are capable of using enzymes to fix nitrogen at ambient soil temperatures (5°C–30°C) and a pressure of about one atmosphere. In addition, enzymes usually exhibit a remarkable specificity for the reactants and reactions, including the ability to distinguish between optical isomers [1].

The Principle of Catalysis

An enzyme, like a catalyst, only increases the rate of a reaction without altering itself at the end of the reaction.

Consider the interconversion of S and P:

$$S \underset{k_b}{\overset{k_f}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} P$$

k_f and k_b are rate constants for the forward and backward reactions respectively. Suppose that, in the absence of an enzyme, the

[1] When a carbon atom has four different substituents, two distinct spatial arrangements are possible, one of which is the mirror image of the other. The two molecules are not super-imposable.Such compounds are called *optical isomers*. In solution, the two isomers rotate the plane of polarised light characteristically in opposite directions.

Box 1. Historical Notes

1833	Wheat amylase purified by Payen and Persoz.
1877	The term enzyme (meaning 'in yeast') coined by Kuhne.
1893	Enzymes act as catalysts shown by Ostwald.
1894	Lock and Key hypothesis put forward by Fischer.
1909	Effects of pH on enzyme action demonstrated by Sorensen.
1913	Theory of enzyme kinetics proposed by Michaelis and Menten.
1926	Urease, first enzyme to be crystallised by Sumner.
1931	Method to determine kinetic constants of enzymes published by Lineweaver and Burk.
1943	Chance proved the existence of enzyme-substrate complexes shown by spectrophotometry.
1960	The primary amino acid sequence of ribonuclease determined by Hirs, Moore and Stein.
1962	The first 3D structure for lysozyme solved by Phillips.
1982	Mutant tryosyl-tRNA synthetase produced by site-directed mutagenesis.
1985	RNA molecules were shown to act as catalysts (ribozymes) by Altman and Cech.
1987	Antibodies raised against transition-state analogs of specific reactions act as enzymes (abzymes).
1990–95	Enzymes with altered substrate specificities /designer enzymes made.

forward rate constant (k_f) is 10^{-4} /second and the backward rate constant (k_b) is 10^{-6}/second, the equilibrium constant K_{eq} is

$$K_{eq} = \frac{[P]}{[S]} = \frac{k_f}{k_b} = \frac{10^{-4}}{10^{-6}} = 100,$$

where $[P]$ is the concentration of the product and $[S]$ is the concentration of the substrate. The equilibrium concentration of P is 100 times that of S even in the absence of an enzyme, but it would take more than an hour to approach this equilibrium, while equilibrium would be attained within a second in the presence of a suitable enzyme.

Enzymes Accelerate the Attainment of the Equilibrium But Do Not Affect its Position

In the presence of a suitable enzyme, both k_f and k_b are enhanced to the same degree. Thus if k_f increases one hundred fold, k_b must also increase one hundred fold.

Box 2. Enzyme Units and Specific Activity

In most preparations, the actual molar concentration of enzyme is unknown. As a result, the amount of enzyme present can be expressed only in terms of its activity. One international unit (IU) of enzyme is that amount that catalyses the formation of 1 μmole of product per minute under defined conditions. The concentration of enzyme in an impure preparation is expressed in terms of units/ml. The *specific activity* of the preparation is expressed as units/mg protein. Specific activities are usually reported for optimal assay conditions at a fixed temperature, with all substrates present at saturating concentrations. Molecular activity or molar activity is the number of moles of substrate transformed per minute per mole of enzyme (units per micromole of enzyme) under optimal conditions.

For the reaction:

$$A + B \underset{k_b}{\overset{k_f}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} C + D,$$

the equilibrium constant K_{eq}, is defined by the equation

$$K_{eq} = \frac{[C][D]}{[A][B]} = \frac{k_f}{k_b}.$$

In the above equation, $[A]$, $[B]$, $[C]$ and $[D]$ represent the concentrations of A, B, C and D respectively and k_f and k_b are the rate constants for the forward and reverse reactions respectively. The catalyst works by accelerating both forward and reverse reactions, and by exactly the same factors. Hence, the final equilibrium mixture resulting from a reaction will be the same, regardless of whether or not it is enzyme catalysed. Before we go through the nitty-gritty details of kinetics, it is useful to understand a few basic concepts.

Transition States and Activation Energy

The transition state is essentially a hypothetical state that cannot be observed directly, because it is defined to be the least populated species along the reaction pathway – that with maximum free energy. The interconversion of two compounds, S and P, during a chemical reaction, involves a transition state (T^*) which has a higher free energy than either type of participating molecule (*Figure 1*). ΔG is the change in free energy of a system undergoing a transformation at constant pressure and temperature. The

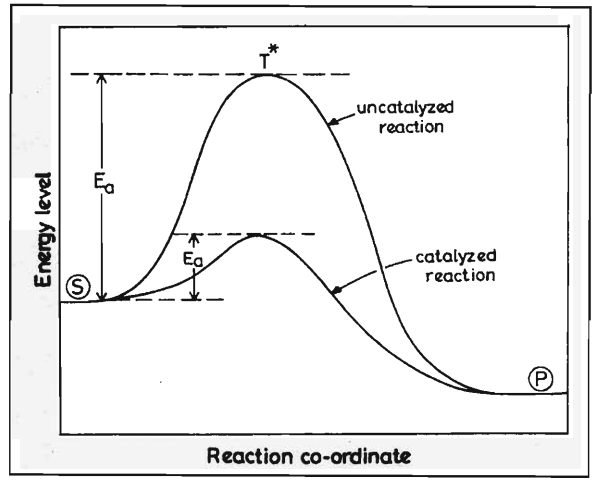

Figure 1. Energy diagram for the reaction S→P in the absence and presence of an enzyme. The uncatalysed reaction for the conversion of S to P via an activated intermediate T* has an activation energy of E_a. Enzyme catalysis of the reaction reduces the activation energy.

difference in free energy between S and the transition state (T^*) is the activation energy (E_a) for the forward reaction.

$$S \rightarrow T^* \rightarrow P.$$

The greater the activation energy, the slower the rate of reaction. While progressing from T^* to P (*Figure 1*), there is no activation energy and hence the rate governing this part of the reaction will be maximal. Hence, in any reaction, the rate of activation of S i.e, $S \rightarrow T^*$ will be the rate limiting step and will dictate the overall rate of conversion of S to P.

Enzymes have no effect on the ΔG of a reaction. If a reaction has very large negative free energy, it does not mean that it will proceed at a rapid rate. For instance, the oxidation of glucose has a ΔG of –686 kcal/mole. In thermodynamic terms, glucose in air is quite unstable. However, glucose as solid crystals does not breakdown to water and carbon dioxide at a measurable rate. In kinetic terms, glucose is quite stable. Before a molecule of substrate can become a molecule of product, it must possess a certain minimum energy in order to pass into T^*. The amount of energy required is called activation energy (E_a). Like all catalysts, enzymes achieve their effect by reducing the free energy of the T^*. Hence, the activation energy is reduced (*Table 1*) and the rate of attainment of equilibrium will be greater in the presence of an enzyme.

Catalyst	Activation energy (J/mol)
None	75,600
Collodium platinum (Chemical catalyst)	49,140
Catalase (Biological catalyst)	8,400

Table 1. The effect of an enzyme on the activation energy for the decomposition of hydrogen peroxide.

Order of a Reaction

A chemical reaction can be classified either according to its order or its molecularity. For example, a reaction $A \rightarrow P$ is unimolecular and a reaction $A+B \rightarrow P$ is bimolecular. Thus, the molecularity defines the number of molecules that are altered in a single reaction step. On the other hand, the order of a reaction defines how many concentration terms must be multiplied together to get an expression for the rate of the reaction. For instance, in a first order reaction, the rate is proportional to the concentration of a single compound. In a second order reaction, it is proportional to the product of two concentrations or to the square of one concentration. An easy way to determine the order of a reaction is to measure the rate (v) at different concentrations of the reactant (S). Then, a plot of log v against log [S] gives a straight line with a slope equal to the order. All first-order rate constants have the dimensions of time^{-1} and second-order rate constants have the dimensions of concentration^{-1}×time^{-1}. Note that a bimolecular reaction need not necessarily appear to be second order under all conditions. Furthermore, the orders of forward and reverse reactions need not be the same.

Consider the reaction: $A+B \rightarrow P$.

The forward reaction could be second order whereas the reverse reaction might be first order. If the concentration of B is far in excess of A, then it is possible that the rate would be independent of the depletion of B and dependent solely on the concentration of A. Under such circumstances, the rate may be described as

first order reaction. There are instances when the reaction order changes as the reaction progresses and such reactions are considered to have mixed order.

The physical significance of the first-order rate constant is that it approximates the fraction of the substrate present that is converted to product per small increment of time. For example, if the first-order rate constant (k) for an enzyme preparation under given experimental conditions is 2.3 min^{-1}, it means that approximately 3.88% of [S] is utilised each second.

$$k = \frac{2.3 \text{min}^{-1}}{60 \text{sec} \times \text{min}^{-1}} = 0.0383 \text{sec}^{-1}$$

Kinetics of Enzyme-Catalysed Reactions

One of the important features of an enzyme-catalysed reaction is that enzymes bring substrate(s) together in favourable orientations in enzyme-substrate (ES) complexes. The substrates bind to the active site in the enzyme, and most enzymes are highly selective in their binding of substrates. The specificity of enzymes indeed depends in part on the specificity of binding. At a constant concentration of enzyme, the reaction rate increases with increasing substrate concentration until a maximum velocity is reached. Uncatalysed reactions do not show this saturation effect. This saturation effect is exhibited by nearly all enzymes. It led Victor Henri in 1903 to conclude that an enzyme combining with its substrate molecule to form an enzyme-substrate complex is a necessary step in enzyme catalysis. In 1913, Leonor Michaelis and Maud Menten interpreted maximal velocity of an enzyme catalysed reaction in terms of the formation of ES complex. At high concentrations of the substrate, the catalytic sites are filled and the reaction rate reaches a maximum. The enzyme will remain unchanged by the reaction and hence will appear on both sides of the chemical equation

$$E + S \underset{k_2}{\overset{k_1}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} ES \underset{k_4}{\overset{k_3}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} E + P,$$

where E is the enzyme, S is the substrate and P is the product. *Figure 2* shows a time course for an enzyme catalysed reaction where the initial reactant concentration $[S_0]$ is much greater than the initial enzyme concentration $[E_t]$.

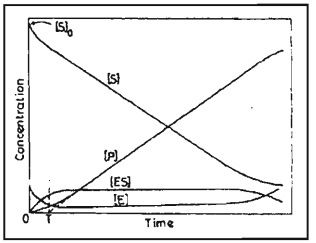

Figure 2. Time course for an enzyme catalysed reaction. The initial substrate concentration $[S]_0$ is significantly greater than the initial enzyme concentration $[E]_t$. As the ratio of $[S]_0/[E]_t$ increases, the steady-state region accounts for an increased fraction of the total reaction time. T represents the presteady-state level.

The existence of an ES complex was inferred from (i) the high degree of substrate specificity exhibited by enzyme, (ii) the shape of the velocity versus substrate concentration curve, and (iii) the fact that substrates often protect the enzyme from inactivation. Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. This model is the simplest one that accounts for the kinetic properties of many enzymes.

Derivation of the Michaelis-Menten Equation

A kinetic equation describing the enzyme-catalysed conversion of S to P (referred to as Michaelis-Menten equation) is derived as follows, provided certain assumptions are made.

1. We restrict ourselves to the initial velocity in the forward direction before a significant concentration of P has accumulated. ($ES \rightarrow E+P$ is assumed to be irreversible).
2. The concentration of ES will remain constant through most of the time taken for the reaction to complete. This is called steady state.
3. $[S] >> [E]$: Under these conditions very shortly after mixing E and S, a steady-state will be established in which the $[ES]$ remains essentially constant with time (*Figure 2*) i.e., rate of ES formation is equal to rate of ES breakdown.

A velocity equation can then be derived:

$$E+S \underset{k_2}{\overset{k_1}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} ES \underset{k_4}{\overset{k_3}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} E+P,$$

The rate of formation of ES is $d[ES]/dt = k_1[E][S]$ and the rate of breakdown of ES is $-d[ES]/dt = k_2[ES] + k_3[ES] = (k_2+k_3)[ES]$. As ES concentration remains constant (second assumption), $d[ES]/dt = -d[ES]/dt$.

Therefore, $k_1[E][S]=(k_2+k_3)[ES]$.
Rearranging,

$$\frac{k_2+k_3}{k_1}=\frac{[E][S]}{[ES]} \qquad (1)$$

The left hand term of equation (1) consisting of the three rate constants is called the Michaelis constant[2], K_m.

[2] The Michaelis constant, K_m is equal to the substrate concentration at which the reaction rate is half its maximal value. It has units of substrate concentration.

$[E]$, concentration of free or unbound enzyme can be rewritten in terms of the total concentration of enzyme E_t and the concentration of the enzyme in the complex $[ES]$.

$$[E] = [E_t] - [ES].$$

Substituting in equation (1), we get,

$$K_m=\frac{k_2+k_3}{k_1}=\left\{\frac{[E_t]-[ES]}{[ES]}\right\}[S]. \qquad (2)$$

Suggested Reading

- I H Segel. Enzymes in *Biochemical Calculations*. John Wiley and Sons Publishers.208–237, 1976.
- A C Bowden. Introduction to Enzyme Kinetics in *Fundamentals of Enzyme Kinetics*.Portland Press. UK Publishers. 19–34, 1995.
- Smith and Wood. Enzyme Kinetics in *Biological Molecules*. Chapman and Hall Publishers.83–94, 1995.
- L Stryer. Enzymes: Basic Concepts and Kinetics in *Biochemistry*. W H Freeman and Company Publications.181–195, 1995.

Hence,

$$K_m=\left(\frac{[E_t]}{[ES]}-1\right)[S].$$

The rate of formation of products or the initial velocity v, of an enzyme catalysed reaction is

$$v = k_3\,[ES]$$

Substituting $[ES]$ in equation (2), we get

$$K_m=\left(\frac{k_3[E_t][S]}{v}\right)-[S]$$

or

$$v=\left(\frac{k_3[E_t][S]}{K_m+[S]}\right). \qquad (3)$$

The maximal velocity that would be observed when all the enzyme

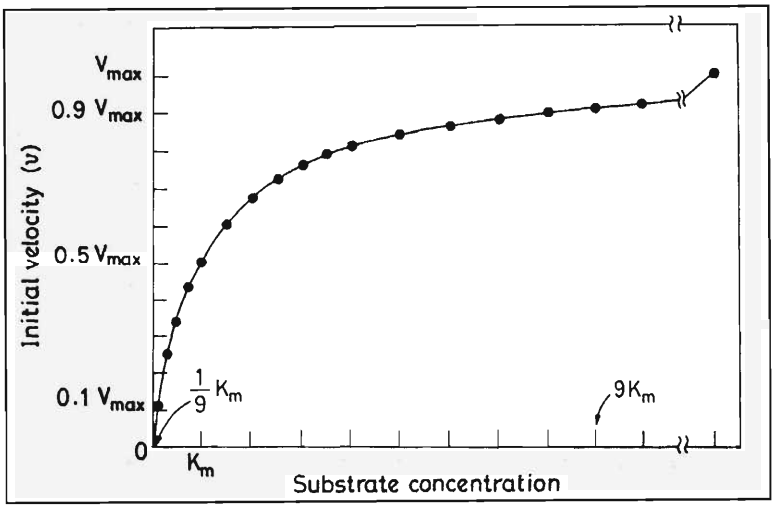

Figure 3. The initial velocity of an enzyme catalysed reaction as a function of substrate concentration.

is present as ES, is given by the following equation

$$V_{max} = k_3 [E_t] .$$

Substituting this in equation (3), we get

$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad (4)$$

which is the *Michaelis-Menten equation*. This equation defines the relationship between initial velocity v and the substrate concentration, $[S]$, as shown in *Figure 3*. On examining the v versus $[S]$ rectangular hyperbola curve, we see three distinct regions where the velocity responds in a characteristic way to increasing substrate concentrations (*Figure 3*). At very low concentrations of substrate, the v versus $[S]$ curve is almost linear and hence velocity is directly proportional to substrate concentration. This is the region of first-order kinetics. At very high concentrations of the substrate, velocity is essentially independent of substrate concentration. This is the region of zero-order kinetics. In a zero-order reaction, the rate is found to be constant, independent of the concentrations of the reactants. At intermediate substrate concentrations, the relationship between v and substrate concentration follows neither first-order nor zero-order kinetics.

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